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ON THE ISSUE OF PREVENTION AND ERADICATION OF MINOR VIRAL BOVINE DISEASES IN UKRAINE

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Summary. The study aimed to evaluate the epizootic status of livestock in Ukraine concerning the prevalence of bovine immunodeficiency virus and bovine foamy virus infections. A literature review was conducted to analyze the epizootic status of livestock farming in various countries regarding bovine immunodeficiency and spumavirus infections. To investigate this issue in Ukrainian livestock, blood samples were collected from 10–15 cows with further DNA extraction and studies via PCR, according to the developers' recommendations. The biological characteristics of bovine foamy virus and bovine immunodeficiency virus were studied by infecting bovine fetal lung (LEK) and calf coronary vessels (KST) cell cultures, with each passage being visually monitored and examined through light microscopy. PCR was performed on the third and fifth passages to detect the genetic material. The genetic material of bovine leukemia virus, bovine immunodeficiency virus, and bovine foamy virus was confirmed in 12 farms across 8 regions of Ukraine. It was demonstrated that bovine immunodeficiency virus and bovine foamy virus can integrate into homologous cell cultures derived from cattle. The immunosuppressive effects of bovine foamy virus and its capability to inhibit components of the non-specific immune system were established on laboratory animal models. Emphasis is placed on the necessity to develop domestic tools for the retrospective diagnosis of bovine immunodeficiency and spumavirus infections and to implement a national anti-epizootic program

Keywords: bovine leukosis, bovine immunodeficiency, bovine spumavirus infection, PCR, immunosuppression, national anti-epizootic program

Introduction. The stability and profitability of the livestock sector in collective farms depend on many factors. Primarily, these include the genetically embedded potential capabilities of the livestock population concerning milk or meat productivity. These potentials, in turn, are realized when the animals are provided with a balanced diet that meets the needs of each age group and productivity direction. An important element ensuring the profitability of livestock farming is the management of animal housing, specifically: the microclimate of livestock buildings, the organization of the work schedule depending on the season, physiological state of individual animals and groups. Perhaps the most critical factor in maintaining a stable and profitable operation in the livestock sector is the health of the herd concerning infectious diseases.

While preventive measures have been developed and implemented against the most particularly dangerous infectious diseases of cattle, there are no specific means developed for minor viral diseases. These minor diseases include *bovine leukosis* (caused by the bovine leukemia virus —BLV), *bovine immunodeficiency* (caused by the bovine immunodeficiency virus — BIV), and *bovine spumavirus infection* (caused by the bovine foamy virus — BFV). Therefore, the system of preventive health measures is limited to general veterinary-sanitary approaches.

A unifying factor for these minor infections in cattle is not only the affiliation of the pathogens to the same Retroviridae family but also the pathological changes caused by their persistence in the bodies of infected animals. Concerning bovine leukosis, it is worth noting that the persistence of the pathogen in the herd and the disease caused by it leads to the loss of the gene pool due to the early culling of valuable breeding and commercial young animals, as well as adult animals. Another significant aspect of the losses is related to the quality deterioration of livestock products: in meat and milk, the protein-fat balance is disrupted. Milk obtained from leukemic animals is prohibited for consumption without prior thermal decontamination.

Special requirements apply to the use of milk from cows with clinical leukosis: according to Ukrainian legislation, such milk, even after thermal treatment, cannot be used for human or animal consumption due to the accumulation of tryptophan metabolites in the product, which are carcinogenic. This milk must be mixed with a disinfectant and then disposed of (SCVMU, 2007).

Another critical element of loss in the livestock sector is the immunosuppressive condition of animals in the stage of infection and clinical manifestation of diseases caused by the pathogens of minor viral infections. It should be noted that the immunosuppressive state hinders the expected immune response of animals to the administration of specific preventive agents, as well as antibiotic and stimulatory treatment methods.

Despite significant costs associated with the use of high-cost preventive and therapeutic measures, their effectiveness in the bodies of animals infected with the pathogens of minor infections decreases by several orders

of magnitude (Gorbatenko et al., 2009; Willems et al., 1993; WOAH, 2018).

It is also important to note that the retrovirus family of pathogens poses a potential medical and social threat because they are structurally similar to the pathogens that cause AIDS and human T-cell leukemia.

If bovine leukemia, which had pandemic characteristics in the recent past, has been reduced to isolated cases of infected animals due to the introduction of diagnostic tools and government programs in most developed countries of the world, particularly in Europe, the eradication program for bovine immunodeficiency and spumavirus infection has not yet achieved the expected results, even though 20–45% of cattle are infected in some countries (Constable et al., 2017; Nuotio et al., 2003; Pinheiro De Oliveira et al., 2013).

The study **aimed** to evaluate the epizootic status of livestock in Ukraine concerning the prevalence of bovine immunodeficiency virus and bovine foamy virus infections.

Materials and methods. A review of literature reports on the epizootic state of animal husbandry in various countries regarding bovine immunodeficiency and spumavirus infections has been conducted. To study a similar issue in Ukrainian livestock sector, blood samples stabilized with anticoagulant (EDTA) were selectively taken from 10–15 cows from livestock farms in the central-eastern region of Ukraine. Molecular genetic methods, specifically polymerase chain reaction (PCR), were used to isolate the genetic material of BFV and BIV. For the detection of BFV proviral DNA, the *Int1-Int2* primer system (external pair, amplified product length of 430 base pairs (bp)) and *Int3-Int4* (internal pair, amplified product length of 221 bp) were used through the 'nested' PCR variant, following the developers' recommendations (Materniak-Kornas et al., 2017). For the detection of BIV proviral DNA, the *RT_+(-)* primer pair was used, flanking a conserved domain of reverse transcriptase (PCR product length 495 bp), as well as the *BIV_Pol_+(-)* primer pair, flanking the *pol* gene of BIV (PCR product length 235 bp). Amplification was carried out using the standard PCR method according to the developers' recommendations (Moody et al., 2002). To detect proviral DNA of BLV, the *BLV-env_3-4* primer pair was used under WOAH recommendations (Fechner et al., 1996), flanking a fragment of the *env* gene of BLV with a length of 444 bp. Reverse transcription and the creation of cDNA were performed using MMLV reverse transcriptase following the manufacturer's instructions. Amplification was performed on a Biometra thermocycler (USA). PCR analysis results were visualized by horizontal gel electrophoresis in a 1.5–2.0% agarose gel.

The biological properties of BFV and BIV were studied using genetic material by infecting two transplanted cell cultures: bovine fetal lung (LEK) and calf coronary vessels (KST). Each passage was monitored daily visually and using light microscopy. In the third and fifth passages, samples were examined using PCR to detect the genetic material of the pathogens.

A study on the biological properties of BFV was conducted using a model of laboratory animals. For this purpose, the experimental group of rabbits (5 individuals) received a single subcutaneous injection of 1 cm^3 of native blood from a donor animal that had confirmed the presence of BFV genetic material. The second group served as the control group. The condition of the experimental rabbits was monitored through visual observation of the animals' viability after infection, as well as conducting hematological, biochemical, and molecular-genetic analyses of blood samples. Blood samples were taken and subjected to analysis every 15 days.

Results. BIV has been identified in many countries worldwide, and co-infection with two or even three minor disease pathogens is common. It is noteworthy that serological studies on bovine immunodeficiency in different countries, based on several scientific publications, reveal a significant prevalence of the disease in the livestock sector globally. For instance, seropositivity in the United States was observed at 4%, in the Netherlands at 1.4%, in Canada at 5.5%, in Germany at 6.6%, and in France at 4%. Immunodeficiency, according to laboratory studies, has also been confirmed in the United Kingdom, Sweden, Costa Rica, Venezuela, New Zealand, and Australia. The percentage of seropositive cattle compared to healthy animals generally ranged between 1–7%. However, in some herds with chronic disease (epizootic persistence), the infection rate reached up to 50%. Among 64% of BIV-seropositive animals with lymphosarcoma, lymphadenopathy, and other disorders, 74% were infected with the immunodeficiency virus (Meas et al., 2002; Rua and Gessain, 2015; Rethwilm and Lindeman, 2013; Rethwilm and Bodem, 2013).

Regarding spumavirus infection, the literature indicates that 30% to 45% of cattle are seropositive for BFV, and the infection caused by it is widespread globally (Materniak et al., 2010, 2013).

According to the results of molecular-genetic studies conducted at the Molecular Diagnostics Laboratory of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine), it was established that in 12 livestock farms across 8 regions, where blood samples from a limited number of animals (10–15 individuals) were selectively examined mostly in farms where anti-leukemia health measures were in their final stages there was evidence of the circulation of BFV, BIV, and associations between the pathogens of spumavirus infection, immunodeficiency, and bovine leukemia. Moreover, in each case, the genetic material of the pathogens, often in associated form, was detected in 20–35% of the samples tested (Fig. 1).

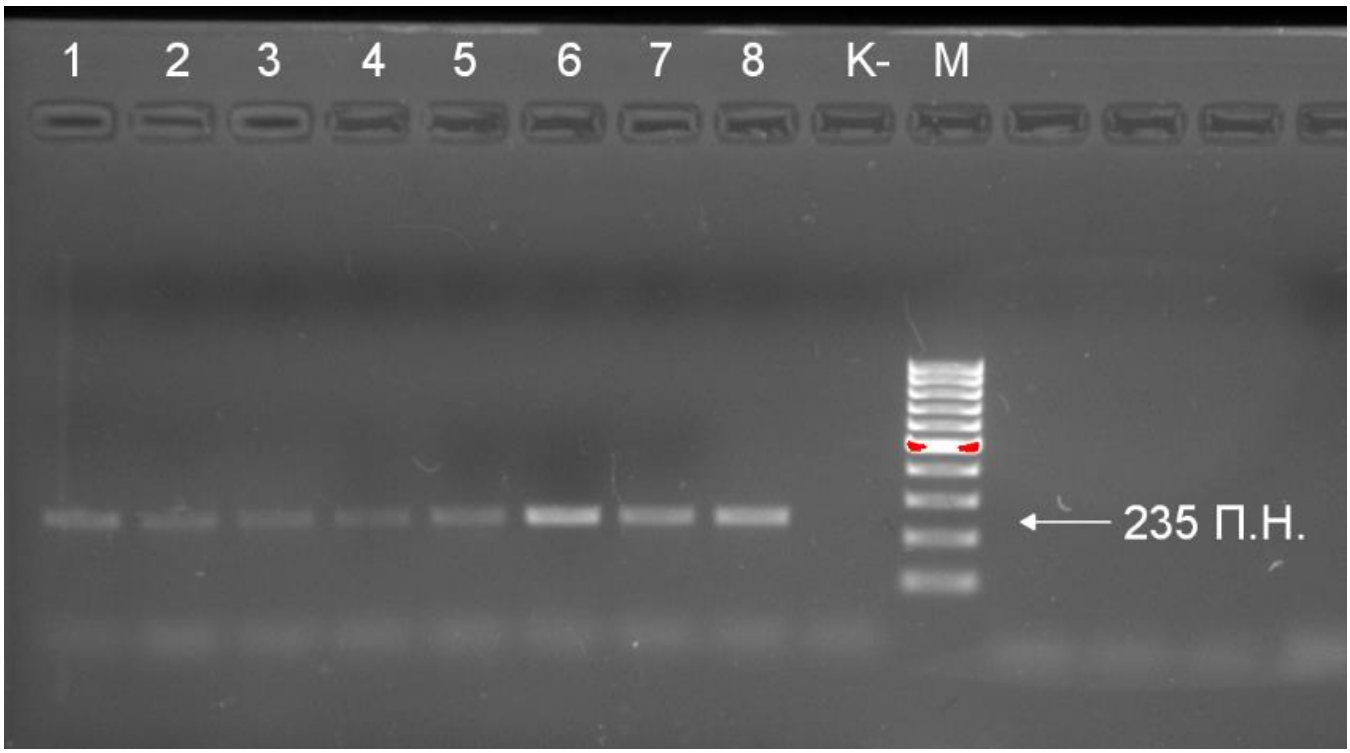

Figure 1. Gel-electrophoresis results of PCR products are as follows: 1–8 — positive clinical samples from cattle infected with BIV; K- — negative control; M — 100 bp molecular weight marker.

Based on the results of testing a limited number of blood samples from each farm's herd, it would be risky to draw conclusions about the infection intensity of the entire herd, taking into account different age groups. From our research, we can only confirm the circulation of slow virus pathogens, namely BLV, BIV, and BFV, in the livestock sector of the examined region of Ukraine.

This data emphasizes the need for further studies aimed at determining the prevalence of these diseases to develop targeted measures for minimizing the damage caused by these diseases to the livestock industry.

Microscopic examinations of LEK and KST cell cultures, conducted after infection, showed that the addition of short-term cultivated lymphocytes did not cause destructive changes in the morphology of both cell lines.

The monolayer cells were densely packed with clearly defined boundaries, and the cytoplasm exhibited a minimal number of vacuoles, while the nuclei retained their typical oval shape (Fig. 2).

Observations of the state of monolayer cell cultures (LEK+BIV) and (LEK+BFV) at 1st, 2nd, and 3rd passages revealed satisfactory coverage of the monolayer. Morphologically, the cells in the experimental cultures were similar to the control cells. PCR results in 3rd passage indicated the presence of genetic material from BIV and BFV in the cells of the monolayer. In 4th to 6th passages, the experimental cell cultures exhibited morphological destruction with signs of syncytium formation — enlarged cells with two or three nuclei were observed. It became more challenging to detach the monolayer cells from the glass using trypsin-versen solution (Fig. 3).

In 7th and 8th passages, the condition of the monolayer remained similar, with a significant increase in the number of dead cells in the culture medium (Fig. 4).

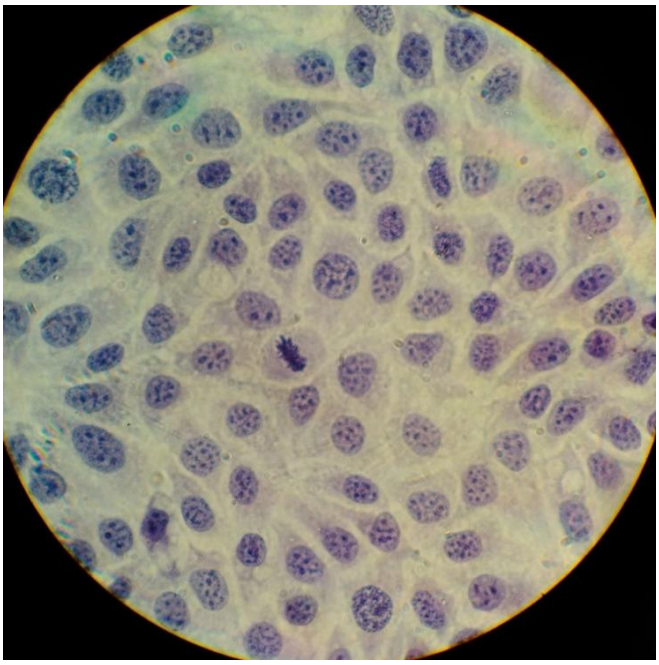

Figure 2. Normal monolayer of LEK cell culture.

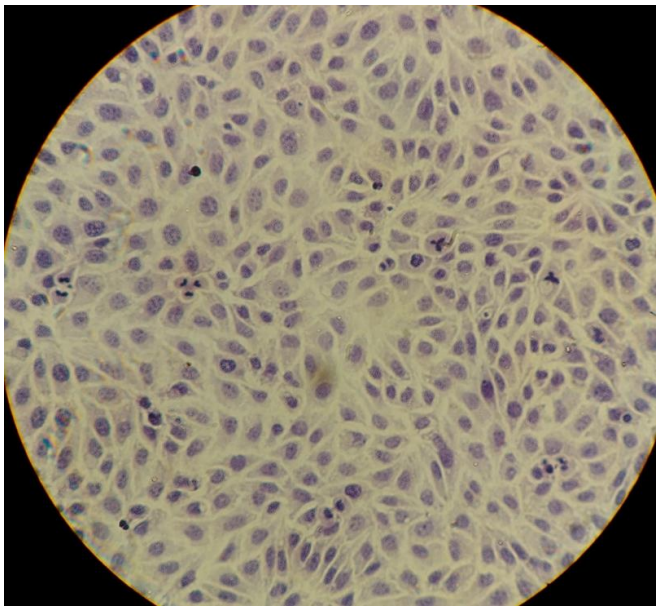

Figure 3. Syncytium formation in the monolayer culture of LEK cells at 4th–6th passages after infection with virus-containing material.

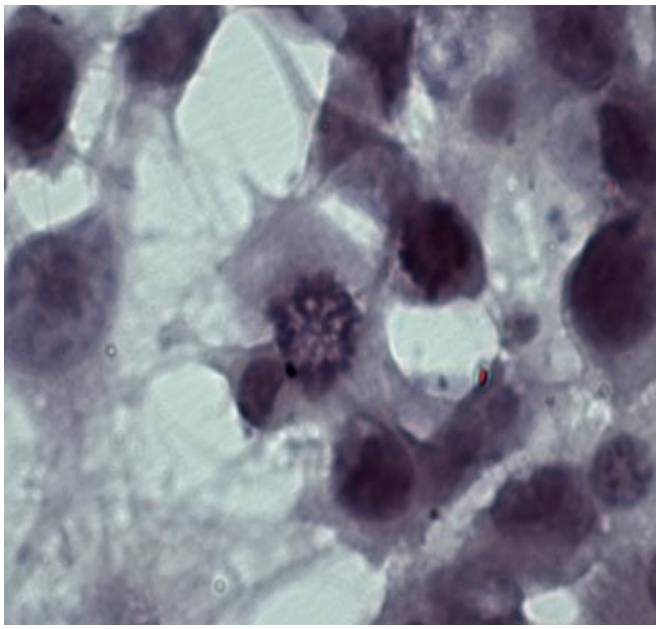

Figure 4. Cell death in the LEK monolayer at 7th–8th passages.

A total of 15 passages were performed for both cultures (LEK+BIV) and (LEK+BFV). The genetic material of BIV and BFV was also detected in the material isolated from the 10th passage. According to the PCR results, the genetic material of the above-mentioned viruses was not detected in the DNA from the cell culture at 13th and 15th passages. The study on the potential integration of field strains of BIV and BFV into the transferrable cell culture of calf coronary vessels (KST) showed a lower sensitivity of this culture to viruses of the Retroviridae family compared to the LEK cell culture.

A total of 7 passages were conducted. At the 5th passage, PCR results still indicated the presence of genetic material from the retroviral pathogens, while the material from the 7th passage yielded a negative result.

Regarding the study of the biological properties of BFV in laboratory animals, it should be noted that molecular-genetic analysis of blood samples from the experimental group of rabbits revealed the presence of BFV genetic material in four out of five rabbits fifteen days after inoculation. After 30 days, positive results for the presence of the genetic material of the mentioned pathogen were found in two rabbits. A third analysis, conducted 45 days after the start of the experiment, yielded similar results as the second analysis, with the genetic material being detected in the blood samples of two experimental animals. By the second month after inoculation (fourth analysis), the genetic material of BFV was found in only one rabbit. It was established that the inoculation of rabbits with the genetic material of BFV causes a short-term persistence of up to 60 days according to molecular-genetic research data. The persistence of BFV virus in rabbits does not cause significant hematological changes, although the redistribution of the leukocyte fraction towards a pronounced lymphocytosis indicates the development of an immunosuppressive state. Experimental infection of rabbits with BFV causes a minor activation of the immune system 30 days after infection, which is followed by pronounced suppression of both functional arms of the nonspecific immune response. The genetic material of BFV causes the manifestation of immunosuppression in rabbits post-inoculation, characterized by leukocytosis and redistribution of the leukocyte fraction towards significant (80–88%) lymphocytosis, a decrease in the concentration of circulating immune complexes, a reduction in globulins, and an increase in serum mucoproteins (Tables 1 and 2).

Discussion. The results of the monitoring studies on the epizootiological situation in Ukrainian livestock concerning minor infections, specifically bovine spumavirus infection and bovine immunodeficiency, conducted using molecular-genetic methodologies, only indicate the presence of issues related to these minor infections without any recommendations for addressing the epizootiological status. Research has widely demonstrated the global presence of BLV in dairy cattle, with significant variation in prevalence depending on geographic region and control measures. For instance, Yang et al. (2019) investigated BLV in Chinese dairy cattle, confirming the circulation of genotype 4 and underscoring the virus's significant impact on herd health due to its association with bovine leukosis, a cancer-causing condition. Studies such as this align with our findings, emphasizing the need for more comprehensive surveillance to control viral infections in livestock.

Table 1 — Dynamics of white blood cell's level (%)

Days of the experiment	Segmented neutrophils		Band neutrophils		Basophils		Lymphocytes	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
15	29.4 ± 2.6	25.4 ± 2.6	4.5 ± 1.2	5.6 ± 1.2	1.4 ± 0.5	1.3 ± 0.2	48.3 ± 4	47.6 ± 4
30	26.6 ± 3.3	23.4 ± 3.3	3.6 ± 1.4	3.8 ± 1.5	0.8 ± 0.4	2.2 ± 0.3	59.1 ± 3	52.2 ± 3
45	19.4 ± 2.6	27.1 ± 1.9	4.5 ± 1.1	4.1 ± 0.7	0.5 ± 0.3	3.2 ± 0.6	67.3 ± 4	49.4 ± 5
60	18.7 ± 2.2	24.6 ± 2.4	5.8 ± 1.4	4.3 ± 1.1	0.8 ± 0.4	2.6 ± 0.5	85.2 ± 5	51.3 ± 4
75	21.2 ± 3.5	26.4 ± 3.7	3.8 ± 1.5	3.2 ± 0.2	0.9 ± 0.4	1.8 ± 0.3	80.6 ± 3	47.6 ± 3
90	23.7 ± 2.3	30.3 ± 2.1	3.3 ± 1.2	4.4 ± 1.2	1.6 ± 0.2	1.6 ± 0.2	81.8 ± 4	49.1 ± 5
105	21.9 ± 3.9	32.5 ± 2.9	3.9 ± 0.7	5.5 ± 1.3	0.8 ± 0.3	1.8 ± 0.3	88.4 ± 3	50.6 ± 2
120	20.8 ± 2.7	33.4 ± 2.7	4.1 ± 1.1	5.9 ± 1.3	1.2 ± 0.4	1.2 ± 0.1	67.2 ± 5	49.4 ± 6

Table 2 — Biochemical parameters of rabbit serum

No.	Total protein, g/L	Albumin, g/L	Globulin, g/L	Circulating immune complexes, mg/mL	Seromucoids, mg/mL
Before the infection					
Experiment					
1	83.1	59.3	23.8	0.15	0.22
2	59.6	44.8	14.8	0.13	0.22
3	76.9	52.4	24.5	0.13	0.25
4	74.8	49.7	25.1	0.11	0.26
5	72.9	51.1	21.8	0.12	0.22
M ± m	73.5 ± 4.7	49.5 ± 4.9	24.0 ± 0.7	0.13 ± 0.01	0.23 ± 0.008
Control					
6	71.2	55.2	16.0	0.11	0.22
7	65.3	45.5	19.8	0.12	0.22
8	75.8	53.1	22.7	0.10	0.23
9	67.0	48.3	18.7	0.10	0.23
10	70.6	54.5	16.1	0.11	0.22
M ± m	69.9 ± 1.2	51.3 ± 1.9	18.7 ± 1.3	0.11 ± 0.004	0.22 ± 0.002
105 days after infection					
Experiment					
1	64.2	45.9	18.3	0.11	0.24
2	61.7	45.3	16.4	0.12	0.30
3	71.2	47.0	24.2	0.11	0.29
4	75.8	50.0	25.8	0.14	0.32
5	70.0	45.3	24.7	0.14	0.32
M ± m	68.6 ± 2.8	46.7 ± 0.9	21.9 ± 1.9	0.124 ± 0.006	0.294 ± 0.016
Control					
6	64.2	45.3	18.9	0.16	0.32
7	79.3	47.0	32.3	0.15	0.26
8	63.2	45.3	17.9	0.18	0.22
9	75.8	46.5	29.3	0.16	0.24
10	78.7	48.2	30.5	0.14	0.21
M ± m	72.2 ± 3.2	46.5 ± 0.6	25.8 ± 2.5	0.158 ± 0.008	0.250 ± 0.022

As for BIV, similar to our findings, this virus has been detected in several cattle populations worldwide. Although BIV is not as thoroughly researched as BLV, its role in immunosuppression and potential to exacerbate other infections is well-established. Bhatia, Patil and Sood (2013) identified BIV's immunosuppressive effects, which contribute to reduced immune responses and increased vulnerability to secondary infections. This echoes our results where BIV's presence correlates with immunosuppressive conditions in cattle.

Bao et al. (2015) studied BFV in cell cultures, particularly its effects on cell morphology and replication dynamics. Similar to our results, BFV was found to cause syncytium formation and other morphological changes in infected cell cultures. BFV, although generally non-pathogenic, has been implicated in the modulation of immune responses in infected animals, a conclusion supported by the observations of immune suppression in our study.

The experimental inoculation of rabbits with BFV in our study aligns with other research investigating retroviral infections in laboratory animals. Rethwilm (2010) examined the persistence of BFV in laboratory animals, demonstrating its short-term persistence and its effects on immune function, including lymphocytosis and leukocytosis. These findings are consistent with those reported in Ukraine, where BFV caused a temporary immune response followed by immunosuppression.

The presence of these minor infections in cattle herds causes significant damage to livestock both directly, by reducing the volume and quality of production, and indirectly, by decreasing the effectiveness of preventive and therapeutic measures due to the immunosuppressive state of infected animals.

A logical task for the near future is to develop a domestic method for retrospective diagnosis of livestock herds to obtain information on the level of infection and develop measures to control and eradicate the epizootic situation. According to the vision of researchers concerned with minor infections, there is a need to accumulate viral material of BFV and BIV and to develop an antigen for the serological identification of infected animals. The molecular-genetic methodology used in our studies does not allow for the examination of the entire herd due to insufficient funding for a comprehensive survey of livestock and monitoring support during the implementation of health programs.

The development of a national serological diagnostic tool for minor viral diseases of cattle, specifically immunodeficiency and spumavirus infections, will enable the creation and implementation of guidelines for their diagnosis and prevention. This will include considerations of transmission pathways, barriers to infection of susceptible individuals, ensuring the quality of livestock products, environmental sanitation, and strategies for preventive and therapeutic programs.

Conclusions. Minor viral diseases of cattle, such as bovine immunodeficiency and spumavirus infections, are widespread in livestock operations worldwide. Selective blood tests from 12 farms in 8 regions of Central and Eastern Ukraine have confirmed the presence of genetic material from BLV, BIV, and BFV viruses, with instances of co-infection. The ability to integrate these pathogens into homologous cell cultures for cattle and the potential for virus mass accumulation have been established. Laboratory animal models have demonstrated that inoculation with spumavirus genetic material leads to immunosuppressive effects and suppression of both branches of nonspecific immunity. Addressing preventive measures and eradicating these viral slow infections, specifically bovine immunodeficiency and spumavirus infections requires the development of domestic retrospective diagnostic tools and the implementation of a national anti-epizootic program.

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